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Effects of Ozone and Water Stress on Canopy Temperature, Water Use, and Water Use Efficiency of Alfalfa

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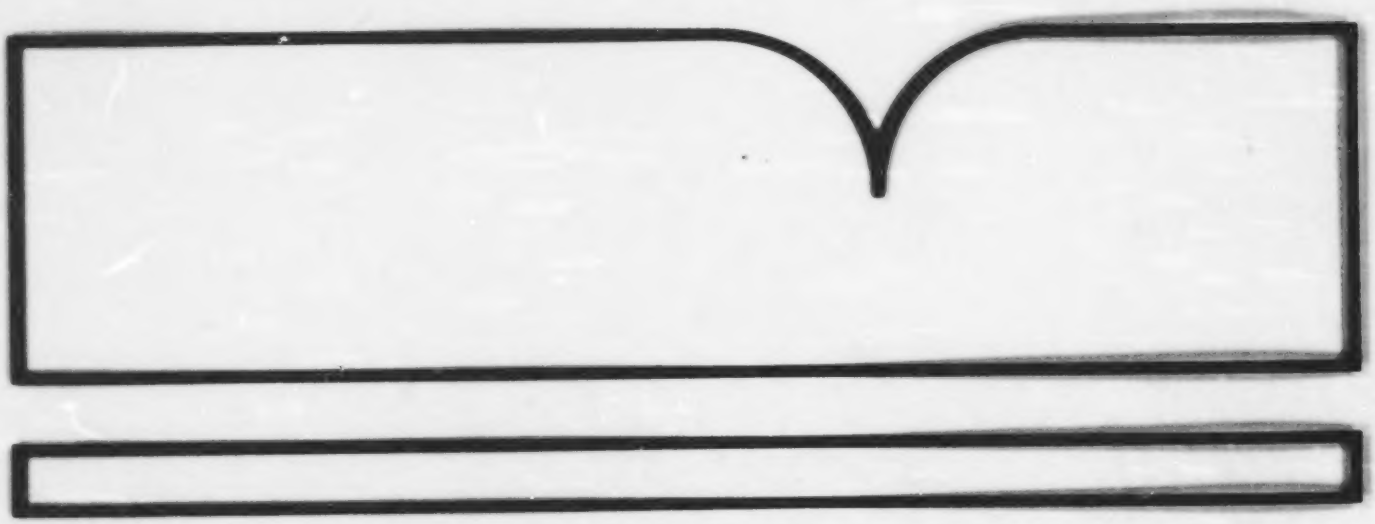

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Ozone(O_3) and soil water deficit are two environmental stresses that significantly affect the growth and yield of alfalfa. However, little is known of the responses of field-grown alfalfa to O_3, and the effects of the interaction between O_3 and water stress on canopy temperature and water relations of alfalfa have not been previously reported. The objective of the study was to determine the interactive effects of O_3 and soil water deficits on canopy temperature, water use and water efficiency (WUE) of alfalfa. Alfalfa was grown in plots of Wasco sandy loam and exposed in open-top chambers to five levels of O_3 (ranging from charcoal-filtered air to twice ambient O_3 concentrations)for 12 h daily. Each plot received either normal or 30% less than normal irrigation. Canopy temperature-air temperature differentials were significantly reduced by water stress. Water use, rate of soil water depletion, or depth of effective rooting zone were not affected by O_3, whereas water stress significantly reduced all three. Water use efficiency was significantly reduced by O_3. The effects of O_3 on WUE were attributed to premature senescence and abscission of older alfalfa leaves.

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Effects of Ozone and Water Stress on Canopy Temperature, Water Use, and Water Use Efficiency of Alfalfa

Patrick J. Temple* and Larry F. Benoit

ABSTRACT

Ozone (O_3) and soil water deficit are two environmental stresses that significantly affect the growth and yield of alfalfa (*Medicago sativa* L). However, little is known of the responses of field-grown alfalfa to O_3, and the effects of the interaction between O_3 and water stress on canopy temperature and water relations of alfalfa have not been previously reported. The objective of this 2-yr study was to determine the interactive effects of O_3 and soil water deficits on canopy temperatures, water use, and water use efficiency (WUE) of alfalfa. Alfalfa (cv. WL-514) was grown in 30-3- by 5.5-m plots on Wasco sandy loam (coarse-loamy, mixed, nonacid, thermic Typic Torriorthents) in Shafter, CA, and was exposed in open-top chambers to five levels of O_3 for 12 h daily, from March to October of 1984 and 1985. Ozone treatments ranged from charcoal-filtered air (CF) to twice ambient O_3 concentrations. Each plot received either normal amounts of irrigation (NI) or 30% less than normal (WS). Canopy temperature-air temperature differentials (T_c–T_a) were significantly reduced by water stress an average of 27.9% in 1984 and 44.0% in 1985. Ozone also significantly reduced $T_c - T_a$ by 31% in NI and 37% in WS plots in 1984, but in 1985 O_3 had no effect on $T_c - T_a$. Water use, rate of soil water depletion, or depth of effective rooting zone were not affected by O_3, whereas water stress significantly reduced all three. Water use efficiency was significantly reduced by O_3, averaging 12% lower in nonfiltered compared with CF plots. The effects of O_3 on WUE were attributed to premature senescence and abscission of older alfalfa leaves.

Additional Index Words: Drought stress, Soil water deficits, *Medicago sativa* L., Irrigation, Evapotranspiration, Crop water stress index, Infrared thermometry.

THE ADVERSE effects of ozone (O_3) on crop growth and yield have been well documented (9). Ambient concentrations of O_3 have reduced yields of alfalfa, cotton (*Gossypium hirsutum* L.), soybean [*Glycine max* (L.) Merr.], wheat (*Triticum aestivum* L.), and other major crops in the United States, resulting in economic losses of hundreds of millions of dollars annually to producers and consumers of agricultural commodities (2). Laboratory studies have shown that exposure to concentrations of O_3 prevalent throughout much of the United States can reduce photosynthesis, transpiration, stomatal conductance, and alter water use efficiency (WUE) of plants (11,12,20,21,22). Few studies on the effects of O_3 on physiological functioning of plants have been conducted in the field, but O_3 has been shown to suppress photosynthesis and reduce stomatal conductance in field-grown snap bean (*Phaseolus vulgaris* L.) (6) and cotton (25). The effects of O_3 on water use and WUE of field-grown crop plants have not been adequately documented (10).

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Plant water status plays a major role in regulating plant response to O_3, primarily through control of stomatal function (25,30,32). Field studies of alfalfa responses to water stress have shown that drought-stressed plants had greater canopy resistance to water loss (33), increased leaf water potential (3,24), elevated canopy temperatures (5,15,17,24), reduced stomatal conductance (5), and reduced stem growth and yield (3,15). The effects of water stress on water use and WUE of alfalfa are not as clearly defined. Donovan and Meek (7) found that WUE decreased in wet and dry irrigation treatments compared with optimally irrigated alfalfa. Reduced WUE in nonirrigated alfalfa was also reported by Carter and Schaeffer (4). However, that study (4) found increased WUE under low irrigation during cooler growing conditions in the fall. Observations of decreased stomatal conductance in alfalfa in response to water stress suggest that drought-stressed plants would be less susceptible to yield losses induced by O_3. The degree to which this protection from O_3 is offset by reduction in carbon fixation induced by the water stress itself has not been determined in the field.

The measurement of plant foliar temperatures has proven to be of considerable practical value for the determination of plant water status in response to soil moisture stress (15,18,23), disease (19), and other environmental stresses (13). Since canopy temperature-ambient air temperature differentials ($T_c - T_a$) are a function of the rate of evaporative cooling of the leaf surface, factors that reduce transpiration, such as water stress or disease, will also reduce $T_c - T_a$. Exposure to O_3 has been shown to reduce transpiration in both laboratory (11,21) and field experiments (25), suggesting the possibility that remote sensing of canopy temperatures may be useful in detecting the effects of O_3 on plants. The objective of this research was to determine the interactive effects of O_3 and water stress on canopy temperatures, water use, and WUE of field-grown alfalfa.

MATERIALS AND METHODS

Thirty plots, each 3 by 5.5 m, were seeded with a non-winter dormant cultivar of alfalfa (WL-514) in August 1983 at the USDA Cotton Research Station in Shafter, CA. The soil is a deep Wasco sandy loam. Prior to planting, a cover crop of cowpea (*Vigna sinensis* L.) was incorporated to a depth of 0.30 m, and superphosphate (0-25-0-10S) was applied at a rate of 140 kg P ha^{-1}. A pre-emergence herbicide (benefin, *N*-butyl-*N*-ethyl-*a,a,a*-trifluoro-2,6-dinitro-*p*-toluidine) was applied at a rate of 7 kg ha^{-1}. Seed of alfalfa was precoated with *Rhizobium meliloti* Dan. and broadcast at a rate of 22.4 kg ha^{-1}. The alfalfa was cut once in December 1983 and once in March 1984 prior to the beginning of treatments. Both O_3 and water stress treatments were distributed randomly among the 30 plots. On 15 Mar. 1984 and 21 to 22 Mar. 1985, open-top chambers (8), 3 m in diameter by 2.4 m high, were centered on a 1-m^2 sample area in 24 of the 30 plots. The six additional plots served as ambient air

(AA) controls. Chambers were equipped with frustra (truncated cones) that restricted top opening to 1.94 m in diameter. Ozone treatments consisted of six charcoal-filtered (CF) and six nonfiltered (NF) chambers, and four chambers each to which O_3 was added in proportion to its concentration in ambient air to provide chamber concentrations equal to 33% (NF × 1.3), 67% (NF × 1.7), or 100% (NF × 2.0) greater than ambient air. Additions of O_3 were proportional to concentrations detected by an O_3 analyzer (Model 1003 AH; Dasibi Environmental Corp., Glendale, CA) sampling ambient air 3 m above ground level. The proportional controller signaled the O_3 generator (Model GEC-1A, Griffin Technics Corp., Lodi, NJ) to supply the required amount of O_3 to maintain O_3 concentrations in the chambers at 33, 67, or 100% greater than ambient. Additions of O_3 started at 0900 h and shut off automatically when ambient O_3 was below 0.03 $\mu L\ L^{-1}$ or at 2100 h. Open-top chamber fans ran from 0630 to 2130 h. Ozone additions began on 16 Mar. 1984 and 23 Mar. 1985 and continued daily, except for harvest days and 2 d after harvest, until 10 Oct. 1984 and 9 Oct. 1985. Ozone dispensing, monitoring, sample line-loss testing, data acquisition, and data quality-assurance procedures have been described previously (27).

Plots within O_3 treatments were assigned at random to two irrigation treatments; normally irrigated (NI) and water-stressed (WS). Volumetric soil water content (θ_v) was measured in each plot by neutron probe (Model 3222; Troxler Electronic Labs, Research Triangle Park, NC) at depths of 0.23, 0.46, and 0.91 m in 1984, and 0.23, 0.40, 0.76, 1.07, 1.68, and 1.98 m in 1985. Soil moisture measurements were taken weekly in the early morning, and also on irrigation days, 2 d post-irrigation (to determine field capacity), and the day after each harvest. The neutron probe was field calibrated by regression of θ_v determined by the probe at depths of 0.23, 0.46, and 0.91 m (0.23, 0.46, and 0.76 m in 1985) with θ_v determined gravimetrically in soil cores from the same depths in each of the 30 plots. Normally irrigated plots were watered when mean θ_v at 0.23 m indicated 50% depletion of available soil water (ASW) from field capacity (175 $mm^3\ m^{-3}$). Irrigation water was applied by flooding the individually diked plots to a depth sufficient to return the NI plots to field capacity. Water-stressed plots were irrigated when mean θ_v at 0.23 m indicated 75% depletion of ASW, or when plants showed symptoms of severe water stress. Water-stressed plots were irrigated with 25% less water than NI plots.

Plant water status was determined by pressure chamber (Model 3000; Soil Moisture Inc., Santa Barbara, CA) measurements of stems with three to four fully expanded leaves between 1100 to 1500 h weekly, or more frequently as the plots dried and plants showed signs of water stress. Readings of −1.2 MPa or lower were considered evidence of water stress (3,24). Ambient temperature-canopy temperature differentials were measured with an infrared thermometer (Model 110: Everest Interscience Inc., Tustin, CA). Duplicate measurements of canopy temperature in each plot were taken at least weekly, in conjunction with neutron probe measurements, near midday, once canopy cover reached 100% (approximately 7 d after the beginning of regrowth). Measurement position was standardized by reference to chamber doors so that the angle (45° from due S) and canopy area (center of sample area) were standardized from plot to plot. In 1985, a sling psychrometer (Weksler Instrument Co., Freeport, NY) was used to measure air temperature and relative humidity 0.50 m above canopy height in each chamber or AA plot immediately before infrared measurements of canopy temperature. Psychrometer measurements were used to check air temperature measurements of the infrared thermometer, and wet bulb depression was used to calculate vapor pressure deficit (VPD) in each chamber or AA plot. These data were used to calculate a crop water stress index (CWSI), according to the methods of Idso et al. (1,14,15).

Plants were harvested from the 1-m^2 sample area when in the 1/10 bloom stage or when crown re-sprouts were 10 to 20 mm long. All plots were harvested the same day. In both 1984 and 1985 there were seven harvests from April to October, at approximately 4-wk intervals. Yield data have been presented elsewhere (26). Water use during each regrowth period was estimated by: $ET = I + P + \Delta S$, where ET is water use, I is irrigation, P is precipitation, and ΔS is the change in stored soil moisture for the regrowth period. Drainage below 2 m was negligible and was ignored for these calculations. Water use efficiency for each plot was calculated as WUE = harvested yield (g dry wt. m^{-2})/water used (mm). Depth of rooting zones was determined by neutron probe measurements of soil water depletion in each plot.

Meteorological data, including potential evapotranspiration (ET_o), were obtained from a USDA meteorological station located 300 m northeast of the field site.

Data on canopy temperatures, water use, and WUE were analyzed by analysis of variance and linear regression. Means separation was at the 5% level of significance, unless otherwise mentioned.

RESULTS

Air Quality and Meteorology

Growing conditions during 1984 and 1985 were typical of the Mediterranean climate of the Central Valley of California. Pan evaporation (ET_o) in 1984 averaged 6.10 mm d^{-1} for the April to October growing period, mean maximum temperature was 31°C, and 11 mm of rain were recorded. In 1985, ET_o averaged 5.77 mm d^{-1}, mean maximum temperature was 30°C, and 35 mm of rain fell during this period (Table 1). Maximum mean monthly ET_o was 8 mm d^{-1} in July of 1984, but in 1985 maximum ET_o occurred in May and June, averaging 7 mm d^{-1} for both months. Ambient O_3 was lower in 1985 than in 1984. Seasonal 12-h (0800–2000 h) O_3 concentrations for the April to October growing season averaged 0.049 $\mu L\ L^{-1}$ in 1984 and 0.042 $\mu L\ L^{-1}$ in 1985, a 14.3% reduction. Seasonal O_3 trends were similar in both years, with the highest mean O_3 concentration occurring in August (Table 1). As O_3 treatments varied in proportion to ambient O_3 concentrations, O_3 concentrations inside chambers followed the same diurnal fluctuations and seasonal trends as ambient O_3.

Table 1. Meteorological and ambient O_3 data for the 1984 and 1985 growing seasons in Shafter, CA.

	1984				1985			
Month	ET_o†	Mean max. temp.	ppt‡	$[O_3]$§	ET_o	Mean max. temp.	ppt	$[O_3]$
	mm d^{-1}	°C	mm	$\mu L\ L^{-1}$	mm d^{-1}	°C	mm	$\mu L\ L^{-1}$
Apr.	4.73	23	5.1	0.037	5.44	27	0	0.037
May	5.86	31	0	0.042	7.09	28	0.5	0.039
June	7.49	32	0.5	0.051	7.10	34	15.5	0.041
July	7.97	37	3.0	0.057	6.57	35	4.3	0.044
Aug.	7.27	35	0.5	0.058	6.07	33	0	0.049
Sept.	5.83	34	0	0.053	4.83	28	10.2	0.045
Oct.	3.52	23	4.6	0.043	3.32	26	4.3	0.039
Season	6.10	31	11.0	0.049	5.77	30	34.8	0.042

† Pan evaporation.
‡ Precipitation.
§ Mean 12-h (0800–2000 h) O_3 concentration for the month.

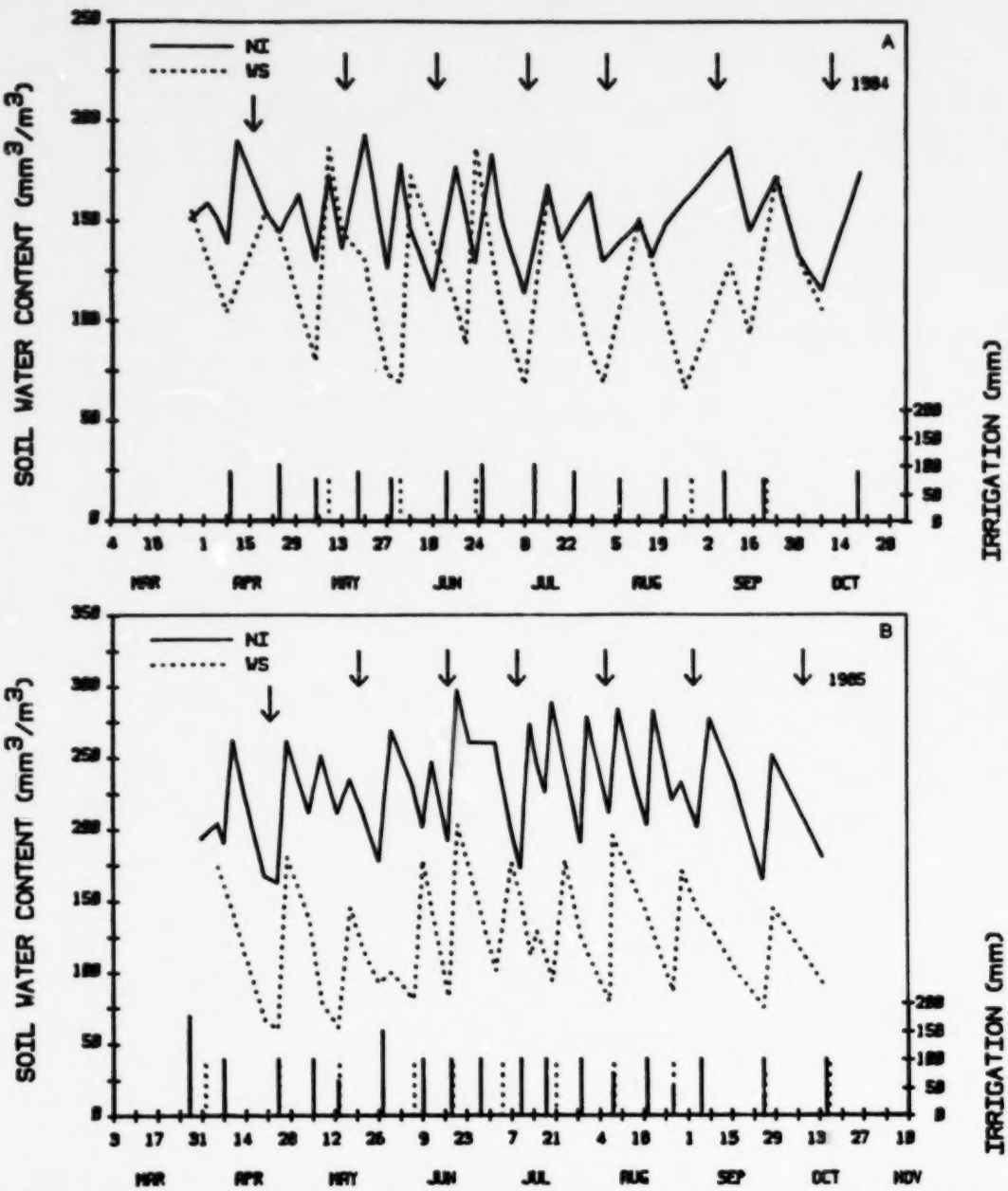
NI
WS
A
1984
SOIL WATER CONTENT (mm³/m³)
IRRIGATION (mm)
4 18 1 15 29 13 27 10 24 8 22 5 19 2 16 30 14 28
MAR APR MAY JUN JUL AUG SEP OCT
NI
WS
B
1985
SOIL WATER CONTENT (mm³/m³)
IRRIGATION (mm)
3 17 31 14 28 12 26 9 23 7 21 4 18 1 15 29 13 27 10
MAR APR MAY JUN JUL AUG SEP OCT NOV

Fig. 1. Volumetric soil water content to a depth of 1 m, dates and amount of irrigation, and harvest dates (arrows) for normally irrigated (NI) and water-stressed (WS) alfalfa plots, March to October, 1984 (A) and March to October, 1985 (B), when soil water content was measured to a depth of 2 m. Each value is a mean of 15 plots.

Soil and Plant Water Status

Soil water levels in NI plots were maintained between field capacity (f.c.) (175 mm^3 for the first meter of soil) and 34% below f.c. in 1984 (Fig. 1). In 1985, maximum soil water levels in NI plots were 10% below f.c. (290 mm^3 for the first 2 m of soil) until June and slightly above f.c. for the remainder of the growing season (Fig. 2). Minimum soil water levels in NI plots averaged 35% less than f.c., although in April and early July soil water in NI plots was depleted to 57% of f.c. Water-stressed plots in both 1984 and 1985 had at least one period of severe soil moisture depletion during each regrowth cycle (Fig. 1). In 1984, maximum soil water depletion in WS plots averaged 53% reduction from f.c. over all the soil water cycles. The fourth (11 June–9 July) and seventh (7 Sept.–10 Oct) regrowth cycles had two drought-stress periods, while other cycles had one, which typically came near the end of the growth period. In 1985, WS plots were stressed more severely than in 1984. Maximum soil

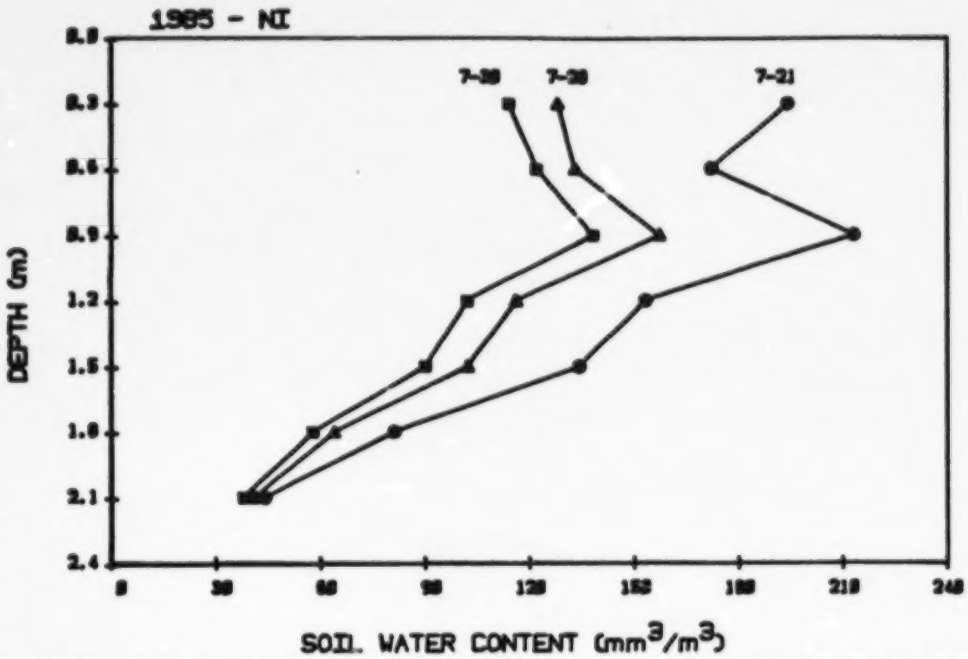

Fig. 2. Soil water depletion to a depth of 2.1 m in normally irrigated (NI) alfalfa plots from 21–30 July 1985. Each value is a mean of 15 plots, averaged across O_3 treatments.

water depletion averaged 69% depletion from f.c. The third, fifth, and seventh cycles regrowth had two drought-stress periods; the first, second, fourth, and sixth regrowth cycles had one each. Water-stressed alfalfa during periods of soil water depletion below 50% of f.c. typically showed xylem pressure potentials of −1.1 to −2.3 MPa (data not shown).

Depth of the rooting zone could not be determined accurately in 1984 because soil moisture was measured only to a depth of 1 m. In 1985 volumetric soil water was measured to 2.1 m in NI plots and 1.8 m in WS plots. Depletion of ASW in the rooting zone is illustrated by measurements of θ_v from the fifth regrowth cycle in July 1985. In NI plots, active depletion of ASW occurred to a depth of 1.8 m, although the bulk of soil water was removed from the first meter (Fig. 2). The root zone in WS plots was more shallow, with 90% of all soil water removed from the first meter of soil (Fig. 3). There was no evidence of an O_3 effect on depth of the rooting zone in NI or WS plots (data not shown).

Table 2. Crop water stress index (CWSI) averaged over O_3 treatments for normally irrigated (NI) and water-stressed (WS) alfalfa in 1985. CWSI can range from 0 (no water stress) to 1 (maximum stress = no transpiration). Each point is the mean of 30 measurements (± 1 SD).

	NI		WS	
Date	Days past irrigation	CWSI	Days past irrigation	CWSI
3 July	5	0.01 ± 0.05	13	0.68 ± 0.07
8 July	10	0.24 ± 0.07	3	0.05 ± 0.04
21 July	2	0.20 ± 0.09	16	0.91 ± 0.04
25 July	6	0.11 ± 0.06	3	0.20 ± 0.05
29 July	10	0.18 ± 0.02	7	0.36 ± 0.07
1 Aug.	2	0.15 ± 0.01	10	0.63 ± 0.08
19 Aug.	10	0.27 ± 0.06	10	0.37 ± 0.07
23 Aug.	3	0.08 ± 0.15	14	0.42 ± 0.06
27 Aug.	7	−0.03 ± 0.12	18	0.84 ± 0.08
20 Sept.	14	−0.07 ± 0.10	24	0.72 ± 0.08
25 Sept.	19	0.16 ± 0.11	29	0.89 ± 0.06
5 Oct.	9	0.07 ± 0.09	9	0.36 ± 0.07

The CWSI was measured 12 times between 3 July and 5 Oct. 1985. On a scale of 0 to 1, where 0 = no water stress and 1 = maximum stress (no transpiration), CWSI for NI plots averaged 0.13 and for WS plots, 0.53. In NI plots, CWSI ranged from −0.03 to a maximum of 0.27, recorded 10 d post-irrigation (Table 2). In WS plots, CWSI ranged from 0.05 (3 d post-irrigation) to a maximum of 0.91 (16 d post-irrigation), indicating almost complete cessation of transpiration. Periods of severe water stress (CWSI >0.50) ranged from 6 to 11 d, depending on timing of irrigations and evaporative demand. There was no evidence of an effect of O_3 on CWSI (Table 4).

Canopy Temperatures

Canopy-ambient air temperature differentials in each plot were measured 19 times between 19 June and 2 Oct. 1984), and 19 times between 13 Apr. and 5 Oct. 1985. In 1984, seasonal mean $T_c - T_a$ for all NI plots averaged −5.3°C, with a range from −7.1 to −3.4°C. Water-stressed plots averaged −3.8°C, with a range from −6.6 to 1.3°C. Analysis of variance indicated that the difference in canopy temperature depression between NI and WS plots was highly significant (Table 4). Ozone also significantly reduced $T_c - T_a$ in both NI and WS plots (Table 3). Regression analyses indicated significant linear relationships between seasonal mean O_3 concentration and seasonal mean T_c

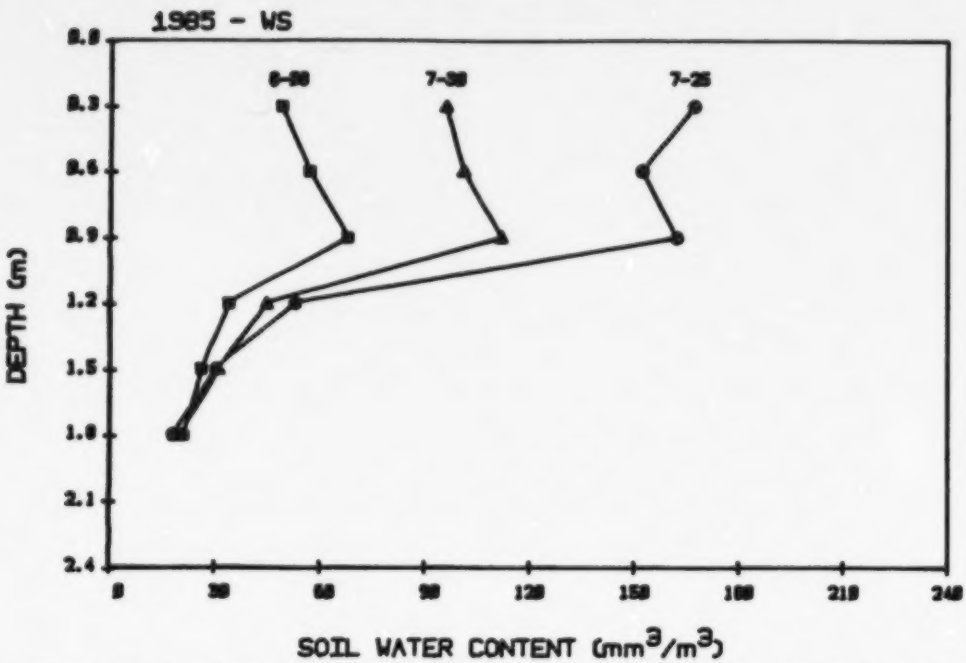

Fig. 3. Soil water depletion to a depth of 1.8 m in water-stressed (WS) alfalfa plots from 21 July–8 Aug. 1985. Each value is a mean of 15 plots, averaged across O_3 treatments.

$- T_a$ in both NI and WS plots. Interaction between O_3 and irrigation levels was not statistically significant. The least-squares best-fit regression equation between $T_c - T_a$ for both NI and WS plots and seasonal 12-h O_3 concentration was

$$Y = -5.1 + 19.79\ (\pm 3.64)\ [O_3] - 1.47\ (\pm 0.22)\ I$$

where $Y = T_c - T_a$, $[O_3]$ is seasonal 12-h O_3 means, and I is a dummy variable in which $I = 1$ if NI plots, and $I = 0$ if WS plots; numbers in parentheses are ± 1 standard error.

In 1985 the difference in mean seasonal $T_c - T_a$ between NI and WS plots was also statistically significant (Table 3). Seasonal mean $T_c - T_a$ for all NI plots was −5.1°C (range −7.1 to −2.9°C), similar to that of 1984. Seasonal mean $T_c - T_a$ for WS plots was lower in 1985 than in 1984, averaging −2.4°C (range −6.8 to 2.3°C). These results were consistent with the soil moisture data, which had also indicated that greater soil moisture depletion had occurred in 1985 than in 1984. However, in 1985 canopy temperature responses to O_3 were not statistically significant in either NI or WS plots (Table 3).

Table 3. Analysis of variance mean squares for seasonal canopy-air temperature differentials for normally irrigated and water-stressed alfalfa exposed to O_3. Canopy-air temperatures measured in each plot on 19 occasions in each year.

		1984		1985	
Source	df	Mean square	F	Mean square	F
Ozone (O)	5	0.91824	8.01**	0.14974	0.08
Irrigation (I)	1	5.4282	47.33**	176.96	93.21**
O × I	5	0.0947	0.83	0.36169	0.19
Error	216				
CV, %			35		55

** Significant at the 0.01 probability level.

Water Use and Water Use Efficiency

Alfalfa water use (ET) in 1984 was underestimated, particularly in NI plots, because soil moisture depletion was measured only to a depth of 1 m. Based on these measurements, seasonal ET in NI and WS plots averaged 1180 and 1080 mm, respectively, In 1985, measurement of soil water depletion to a depth of 2.1 m indicated that alfalfa in NI and WS plots extracted 62 and 94%, respectively, of soil water from the first meter of the soil profile. Assuming that the proportion of water used from the second meter of soil in 1984

Table 4. Seasonal 12-h (0800–2000 h) mean O_3 concentrations, seasonal mean canopy-air temperature differentials ($T_c - T_a$), and seasonal mean crop water stress indices (CWSI) for normally irrigated (NI) and water-stressed (WS) alfalfa; means of 19 daily measurement ($T_c - T_a$) between 19 June and 2 Oct. 1984 and 13 Apr. and 5 Oct. 1985, or 12 daily measurements (CWSI) between 3 July and 5 Oct. 1985.

	1984			1985				
		$T_c - T_a$			$T_c - T_a$		CWSI	
Treatment†	$[O_3]$	NI	WS	$[O_3]$	NI	WS	NI	WS
	μL L⁻¹			μL L⁻¹				
CF	0.016	−6.1	−4.3	0.010	−5.1	−2.5	0.10	0.54
NF	0.045	−5.8	−4.1	0.038	−5.1	−2.2	0.10	0.55
NF × 1.3	0.073	−5.3	−3.3	0.063	−5.2	−2.3	0.10	0.53
NF × 1.7	0.091	−4.6	−3.7	0.078	−5.1	−2.5	0.11	0.53
NF × 2.0	0.109	−4.2	−2.7	0.094	−4.8	−2.5	0.10	0.51
AA	0.049	−5.8	−4.9	0.042	−5.1	−2.7	0.19	0.51

† Treatment abbreviations are explained in the text.

Table 5. Total water use (mm) of normally irrigated (NI) and water-stressed (WS) alfalfa exposed to O_3 in Shafter, CA, 1985, for each exposure period.

Treatment†		Harvest date						
		23 Apr.	21 May	18 June	10 July	7 Aug.	4 Sept.	9 Oct.
CF	NI	192.5	174.2	236.2	253.0	316.8	284.4	228.1
	WS	165.0	158.5	173.9	213.3	194.9	199.3	121.4
NF	NI	211.4	185.4	228.7	265.2	334.2	298.2	238.3
	WS	166.4	171.1	219.9	216.0	227.9	204.4	144.2
NF × 1.3	NI	211.4	173.0	216.8	260.8	315.0	279.3	239.6
	WS	162.5	171.7	198.8	223.8	221.9	201.2	141.1
NF × 1.7	NI	202.7	191.3	226.1	269.1	319.1	289.8	241.1
	WS	172.6	196.1	198.9	218.4	232.8	202.6	139.2
NF × 2.0	NI	206.0	159.7	221.1	265.2	315.5	292.7	245.5
	WS	154.1	158.3	201.7	216.2	217.5	196.0	131.6
AA	NI	204.8	178.3	256.8	266.5	348.6	306.4	251.4
	WS	173.7	184.0	204.0	235.0	222.9	208.5	155.6

† Treatment abbreviations are explained in the text. Means of three CF, NF, and AA plots; two NF × 1.3, NF × 1.7, and NF × 2.0 plots.

Table 6. Seasonal mean 12-h (0800–2000 h) O_3 concentrations, water use (ET), and water use efficiency (WUE) of normally irrigated (NI) and water-stressed (WS) alfalfa exposed to O_3 from 16 Mar. to 10 Oct. 1984 and from 23 Mar. to 9 Oct. 1985.

Treatment†	$[O_3]$	NI		WS	
		ET	WUE	ET	WUE
	$\mu L\ L^{-1}$	mm	$g\ m^{-2}\ mm^{-1}$	mm	$g\ m^{-2}\ mm^{-1}$
		1984			
CF	0.016	1210	2.42	1060	2.42
NF	0.045	1240	2.25	1170	1.91
NF × 1.3	0.073	1140	2.13	1150	1.76
NF × 1.7	0.091	1320	1.59	920	2.16
NF × 2.0	0.109	1030	1.86	1050	1.66
AA	0.049	1130	2.55	1100	2.35
		1985			
CF	0.010	1690	1.72	1230	1.62
NF	0.038	1760	1.69	1350	1.39
NF × 1.3	0.063	1700	1.45	1320	1.40
NF × 1.7	0.078	1740	1.26	1360	1.27
NF × 2.0	0.094	1710	1.27	1280	1.28
AA	0.042	1810	1.65	1380	1.61

† Treatment abbreviations are explained in the text.

was the same as in 1985, seasonal ET in 1984, corrected for missing data below 1 m, was 1900 mm in NI and 1150 mm in WS plots. Water use in NI plots averaged 9.1 mm d^{-1}, and ET in WS plots averaged 5.5 mm d^{-1}, compared with an average ET_o of 6.1 mm d^{-1}. In 1985, ET in NI plots totaled 1740 mm for the season and averaged 8.7 mm d^{-1}. Water-stressed plots' ET totaled 1320 mm and averaged 6.6 mm d^{-1}, compared with a seasonal mean ET_o of 5.7 mm d^{-1}. Water use in 1985 increased during the summer and reached its maximum in July (Table 5); however, ET did not significantly correlate with ET_o, which reached its maximum in May and June. Exposure to O_3 had no apparent effect on ET, as correlation between seasonal mean O_3 concentration and seasonal ET for both NI and WS plots was not significant (Table 6). Correlation between exposure to O_3 and ET for individual harvests (Table 5) also was not statistically significant. Water use in AA plots was about 5% greater than in chambered plots, but this difference was not statistically significant.

Water use efficiency for the seven harvests in 1985 is shown in Table 7. Although WUE was about 10% higher in NI than in WS plots for most of the growing season, in April and October, when ET_o was relatively low, WUE in WS alfalfa was 6.6% higher than in NI plots. Water use efficiency for both soil water regimes peaked in May, which was also the period of maximum ET_o (Table 1). Regression equations between seasonal WUE and seasonal 12-h O_3 concentrations are given in Table 8. In 1984, WUE in NI plots was significantly negatively correlated with O_3 dose, but correlation between WUE and O_3 was not significant in WS plots. Each 0.01 $\mu L\ L^{-1}$ rise in seasonal 12-h O_3 concentrations reduced WUE in NI plots by 3.8%. In 1985, only the O_3 term in the regression model was statistically significant, indicating that neither the irrigation level nor the irrigation × O_3 interaction significantly affected WUE. Thus, NI and WS plots were similar in response of WUE to O_3 in 1985. Each 0.01 $\mu L\ L^{-1}$ increase in seasonal O_3 dose decreased WUE in NI and WS plots 3.5%, similar to the 3.8% decrease observed in NI plots in 1984.

DISCUSSION

Water stress and O_3 can have both complementary and antagonistic effects on plants. Both water stress and O_3 can close stomates and reduce transpiration (5,21,25); thus, both stresses should elevate canopy temperatures. However, water stress and O_3 can have opposite effects on plant architecture. Plants respond to prolonged water stress by reductions in leaf area and by a proportional increase in root growth, so that shoot/root ratios (SRR) decrease. Conversely, plant growth responses to O_3 are often first observed as reduced root growth, so that O_3-stressed plants show increased SRR (29). While reduced root growth in response to O_3 has been reported in laboratory and greenhouse studies, this response to O_3 has not been adequately documented in the field. If plants injured by O_3 have reduced transpiration and reduced root growth, one would expect that these plants would also have lowered water use. The effects of O_3 on WUE are more difficult to predict because, if O_3 reduces growth as well as ET, WUE will depend on the balance between inhibition of growth and reduced water use. This 2-yr study of the responses of field-grown alfalfa to the combined stresses of O_3 and soil water deficiency documented reductions in $T_c - T_a$ in response to both stresses, reductions in ET in response to water stress but not O_3, reductions in WUE in response to O_3, an increase in WUE in WS plants under condi-

Table 7. Mean water use efficiency (g m^{-2} mm^{-1} H_2O) of normally irrigated (NI) and water-stressed (WS) alfalfa exposed to O_3 in Shafter, CA, 1985, for each exposure period.

Treatment†		Harvest date						
		23 Apr.	21 May	18 June	10 July	7 Aug.	4 Sept.	9 Oct.
CF	NI	1.97	3.86	2.05	1.67	1.34	1.27	1.60
	WS	2.10	2.13	1.77	1.39	1.30	1.22	1.74
NF	NI	1.72	2.76	2.14	1.52	1.30	1.31	1.45
	WS	1.85	1.99	1.66	1.41	1.05	0.99	1.29
NF × 1.3	NI	1.32	2.36	1.93	1.50	1.12	1.12	1.31
	WS	1.72	1.98	1.69	1.39	0.98	1.04	1.15
NF × 1.7	NI	1.48	2.13	1.68	1.18	0.94	0.86	1.04
	WS	1.47	1.67	1.52	1.29	0.85	0.98	1.23
NF × 2.0	NI	1.39	2.42	1.69	1.27	0.96	0.95	1.07
	WS	1.53	1.92	1.44	1.21	0.87	0.86	1.48
AA	NI	2.27	2.87	1.84	1.63	1.25	1.36	1.20
	WS	2.16	2.02	1.78	1.35	1.35	1.29	1.31

† Treatment abbreviations are explained in the text. Means of three CF, NF, and AA plots; two NF × 1.3, NF × 1.7, and NF × 2.0 plots.

tions of low evaporative demand, and a decrease in WUE during periods of high ET_o.

Canopy temperature was a sensitive indicator of plant water status in both years of this experiment, confirming the applicability of infrared thermometry in general, and the CWSI in particular, as an indicator of drought stress (1,14,18). In 1984, O_3 also significantly reduced $T_c - T_a$, but this response was not observed in 1985. The reasons for this difference are not apparent. Growing season conditions in 1985 were slightly cooler and more humid than in 1984, and remote sensing of canopy temperatures is not as sensitive under humid conditions as under intense ET_o (15), but differences in ET_o between the 2 yr averaged only 5%. Ambient O_3 concentrations were also about 14% lower in 1985 than in 1984, but the effects of O_3, in terms of reductions in yield, were about the same in the 2 yr (26), so the lack of response of $T_c - T_a$ to O_3 observed in 1985 was not attributable to lack of plant response to O_3 in general. The effects of O_3 on $T_c - T_a$ observed in 1984 were presumably due to reduced transpiration of O_3-injured plants. Reductions in transpiration of alfalfa exposed to O_3 were measured by steady-state porometry, and a significant correlation between reduction in transpiration and increased exposure to O_3 was observed (28). This correlation was not observed in 1985 (unpublished data). Severe foliar injury and significant reductions in growth of alfalfa were observed in both 1984 and 1985 (26), yet alterations in $T_c - T_a$ were documented only in 1984. This suggests that remote sensing of canopy temperatures may hold some potential for the detection of O_3 effects on plants in the field, but the failure of canopy temperature measurements to detect significant O_3 effects in 1985 suggests that such measurements should be used with extreme caution.

The reduction in ET and WUE of alfalfa in response to drought stress observed in this study was similar to that reported for previous field studies of the effects of soil moisture stress on alfalfa (4,7). Water use rates averaged 9.1 mm d^{-1} for NI and 6.6 mm d^{-1} for WS plots in 1985, similar to the mid-summer rates of 5.3 to 10.0 mm d^{-1} reported for field-grown alfalfa in Minnesota by Carter and Schaeffer (4). This is significant because it suggests that the water relations of alfalfa grown in open-top chambers was similar to that of other small-plot alfalfa experiments. Carter and Schaeffer (4) reported highest WUE for alfalfa given "medium low" irrigation when harvested in October, compared with an August harvest in which alfalfa watered at the highest rate of irrigation had the highest WUE. We found that WS alfalfa had higher WUE than NI alfalfa in the April and October harvests, while the reverse was true for the rest of the summer. Since relative ET in NI and WS plots did not vary among harvest intervals, these differences in WUE were attributable to greater relative growth of WS alfalfa compared with NI alfalfa during periods of low ET_o (26).

Contrary to expectations, O_3 had no apparent effect on ET, but O_3 exposure significantly reduced WUE in both years of the study. The lack of measurable effect of O_3 on ET (despite measurement of reduced rates of transpiration in O_3-injured plants, particularly at high O_3 concentrations) may have been due to increases in evaporation in chambers containing O_3-injured plants. Premature senescence and abscission of lower leaves was commonly observed in high O_3 treatments following 2 to 3 wk of exposure to O_3. Premature senescence and foliar abscission have been frequently observed in plants in response to O_3 exposure, possibly in response to increased production of ethylene (31). Higher rates of evaporation from the exposed soil surface may have balanced reduced transpiration from these O_3-injured plants so that ET did not vary with O_3 treatment. Reductions in WUE at higher O_3 concentrations may also be attributable to premature senescence and abscission of O_3-injured leaves. These older leaves would have used water during early growth, but because of leaf abscission they

Table 8. Regression equations between seasonal 12-h O_3 means (μL L^{-1}) and seasonal mean water use efficiency (WUE) (in g m^{-2} mm^{-1}) of normally irrigated (NI) and water-stressed (WS) alfalfa, grown in Shafter, CA, 1984 and 1985; numbers in parentheses are ±1 SE.

1984—NI	
$Y = 2.682 - 8.599\ [O_3]$ (±3.071)	$F = 7.84^*$ $r^2 = 0.66$
1984—WS	
$Y = 2.454 - 6.428\ [O_3]$ (±3.357)	NS
1985—Combined NI and WS	
$Y = 1.762 - 5.435\ [O_3]$ (±0.967)	$F = 31.60^{**}$ $r^2 = 0.76$

*,** Significant at the 0.05 and 0.01 probability levels, respectively. NS = not significant.

would not have added to harvest weight because only fresh, green leaf and stem material was collected at harvest. Thus reductions in WUE in O_3-stressed alfalfa may be attributable to premature leaf senescence and abscission. Reduced WUE (defined as grams of CO_2 fixed/grams of H_2O transpired) was observed in soybean in response to elevated O_3 concentrations in a growth chamber experiment (21). This reduction was attributed to an O_3-induced reduction in net photosynthesis rather than a direct effect of O_3 on stomatal conductance. Ozone has been shown to inhibit photosynthesis in numerous plant species, even in leaves lacking evidence of visible foliar injury (11,20,21,22). Ozone-induced inhibition of photosynthesis was measured in the alfalfa used in this study (28). This suggests that exposure to O_3 reduced photosynthesis in alfalfa, leading to increased internal CO_2 concentrations, which induced stomatal closure and reduced rates of transpiration. However, these lower transpiration rates did not lead to increased WUE because leaf abscission reduced harvested mass more than lower transpiration reduced ET, so WUE was lower in the higher O_3 treatments. Although water stress also reduced transpiration, as evidenced by reduced $T_c - T_a$ in WS plots and by direct measurement of rates of stomatal conductance and transpiration (28), drought stress did not significantly alter alfalfa responses to O_3. Reich et al. (21) also found no interaction between water stress and O_3 on stomatal behavior of soybean. It is possible that a significant interaction between water stress and O_3 would have occurred had growing conditions been more extreme and more severe water stress had developed (27).

The impact of O_3 on root growth was not measured directly in this experiment, but some information can be obtained on the effects of O_3 on root function by measurement of the rate at which water was removed from the soil profile in each plot. We found no evidence that NI or WS alfalfa exposed to high concentrations of O_3 differed from control plants, either in depth of root zone or in amount of water extracted from the soil. While this evidence does not contradict published reports of reduced root growth in response to O_3 (29), it suggests that application of this observation to field-grown alfalfa should be approached with caution. Much of the previous research on the effects of O_3 on physiological function and growth of crop plants has been conducted on annuals, particularly soybean. The present experiment on alfalfa, a deeply rooted perennial plant with numerous vegetative harvests, has supported some, but not all, aspects of the model of O_3 effects on plants generated from research on annuals. In particular, models of $O_3 \times$ drought stress interactions developed for soybean, which assume reduced root growth and water use in O_3-injured plants (16), may not be applicable to alfalfa.

CONCLUSIONS

This 2-yr study of the combined effects of O_3 and soil moisture deficits on canopy temperature and water relations of alfalfa documented reduced $T_c - T_a$ in response to both stresses, reduced ET in response to water stress but not O_3, and reductions in WUE in response to O_3. No statistically significant interactions between O_3 and water stress were found in this study. Canopy temperature was a sensitive indicator of plant water status, particularly when used to calculate the CWSI. Exposure to O_3 significantly increased canopy temperatures in 1984, presumably by reductions in transpiration, but O_3 had no demonstrable effect on canopy temperature in 1985.

Water stress reduced depth of soil moisture extraction of alfalfa, but O_3 had no significant effect on rate or depth of soil moisture extraction either in NI or WS plots. Water stress reduced ET and WUE during periods of high evaporative demand, but in April and October, in response to relatively low ET_o, WUE was greater in WS than in NI plots. Ozone had no effect on ET, but O_3 significantly reduced WUE in NI plots in both years of the study and in WS plots in 1985. This effect of O_3 on WUE was attributed to premature senescence and leaf abscission of O_3-injured leaves. This reduction of WUE by O_3 suggests that in addition to the direct effect of O_3 on yield reduction, O_3 can have an indirect effect by increasing the cost of production of this forage crop.

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